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Physical Basis for Separation of Rice Starch using Various Density Gradient Systems and its Effect on Starch Recovery, Purity, and Pasting Properties

A 32% waxy and non waxy rice flour slurry containing starch-protein agglomerates was physically disrupted in presence of water by use of high pressure homogenizer called Microfluidizer®. Microfluidized rice flour slurry from passes 2 and 4 was collected followed by isolation of starch using three different density gradient solutions/systems (CeCl, NaCl/sucrose and $ZnSO_4 \cdot 7H_2O$). Complete deagglomeration was obtained after passing the rice flour slurry four times through the Microfluidizer®. The recovery of isolated starch varied from 76.28% to 91.20% for different density gradient systems. The degree of deagglomeration did not seem to affect recovery but affected the purity of the isolated starches. All starches produced from pass 4 rice slurry resulted in starches with residual protein below 0.5%. Higher density of the gradient solution resulted in higher recovery and purity of starch. The isolation method had a significant effect on the pasting properties of the isolated starch. Residual protein in isolated starch had a negative correlation with peak viscosity and setback of pass 2 waxy and non-waxy starches. The salts were retained in purified starch despite rigorous washing (at least 75 times greater than the control) and could affect starch properties.

Keywords: Rice starch; Wet milling; Purification; Density gradient; Separation

Research Paper

1 Introduction

Rice starch is commercially purified for use by the food industry. Laboratory extraction methods are usually used to develop commercial methods or to select varieties for introduction into common use. Commercial and laboratory methods of isolating starches can be separated into two important steps. The starch is first deagglomerated from the protein and then it is purified or washed. These methods of deagglomeration of starch-protein agglomerates or washing can impact the physico-chemical properties of starch.

Numerous methods of manufacture of starch on commercial and laboratory scale are discussed and summarized in the literature [1, 2]. They can be categorized into two types. One set of methods uses chemicals to break the protein-starch agglomerates and the others use physical forces. It is extremely important to determine if complete deagglomeration has been achieved by any of these processes. Higher amounts of agglomerates will increase the residual protein content in the starch and thus impact the physico-chemical properties of the starch. Chemical methods, which solubilize the protein, are easier to use, because starch and protein can be easily separated. Physico-chemical properties of starches can, however, be altered by the chemical methods used for deagglomeration [2,3]. Therefore, it is important to select methods of deagglomeration which do not alter starch properties.

Limited information is available on the effect of methods of washing on the starch properties. A review of the methods [4] for studying purification and separation of biological components led us to believe that the capability of commercial starch washing systems and efficiency of method of deagglomeration to achieve starch purity (<0.5% protein) could be tested using density gradient separation. If starch could be purified using a laboratory method with high recovery, then it could be possible to scale up the deagglomeration method to a commercial system.

A density gradient is usually formed by adding an additive to a centrifugation medium such that a true solution is formed. The additive does not interfere with or damage the sample and is compatible with the sample. The resulting solution must have a refractive index within the practical range and the additive must be easily removable from the sample. Additives used are usually divided into four

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main categories: salts of alkali metals, neutral water soluble molecules (sucrose, glycerol), hydrophilic molecules (dextran) and synthetic molecules (epichlorohydrin copolymers). Among these, sucrose and salts have been used to purify starches [5, 6].

A novel method of deagglomeration of the rice starch-protein agglomerates by physical disruption in presence of water was developed [1]. This was accomplished by use of a high pressure homogenizer called Microfluidizer® followed by density based separation. It was determined that 32% slurry and two passes through the homogenizer were optimum and that the optimum pressures for non-waxy rice and waxy flour were 10.0×10^4 kPa and 6.2×10^4 kPa , respectively. The rice slurry was centrifuged and washed twice with water. These conditions yielded low-protein starch with low starch damage (5.5 to 6.3%). It was assumed that when the particle size of the microfluidized slurry was less than 10 μm, a complete deagglomeration was obtained but the study did not address the possible production of purified starches with less than 0.5% protein with this method of deagglomeration.

Our goal was to determine the efficiency of the Microfluidizer® on the deagglomeration of rice flour and to compare the effect of various density gradient systems on separation of rice starch and protein. The cost of the additives used to make density gradient systems, recovery and purity of the recovered starch was evaluated, as well as the efficiency of various density gradient systems. The effect of using various salts in density gradient solutions on the starch pasting properties was also determined.

2 Materials and Methods

Waxy and non-waxy rice flours (RF-W1120 and RF-L0120) were obtained from Sage V Foods, Freeport, Texas. The protein content of the flours were 7.17% and 9.07%, respectively. The Microfluidizer® (Model 110-T, MFIC Corporation, Newton, Massachusetts) was equipped with two chambers: F20y and H230Z. A 32% waxy and non-waxy flour in water was passed four times through the Microfluidizer® at 10.0×104 kPa. Samples were collected from passes two and four for further analysis. Experiments were conducted in triplicate. Rice slurry samples from the microfluidizer were collected in centrifuge tubes (250 mL capacity) and centrifuged at 14,000 $\times$ *g* for 7 min. The microfluidized rice slurry was separated into two fractions, namely, protein and starch. The protein fraction was on top and was scraped off and discarded along with the supernatant after each wash. The starch fraction in the centrifuged tube was re-suspended and re-centrifuged using four different density gradient protocols as follows: (1) re-suspension and re-centrifugation twice in 80% (w/v) CeCl (Sigma-Aldrich, St. Louis, MO) followed by re-suspension and re-centrifugation with water four times; (2)re-suspension and re-centrifugation once in 4 M NaCl (J. T. Baker, Phillipsburg, NJ), then repeating in 6 M NaCl /50% (w/v) sucrose (J. T. Baker, Phillipsburg, NJ) followed by re-suspension and re-centrifugation with water four times, (3) re-suspension and re-centrifugation twice in 75% (w/v) $ZnSO_4 \cdot 7H_2O$(Sigma-Aldrich, St. Louis, MO) followed by re-suspension and re-centrifugation with water four times; (4) re-suspension and re-centrifugation in water six times. After the last centrifugation and scraping, the starch was dried at 50 °C in a hot air convection oven for 24 h. Starch and protein recovery was determined based on the total amount of starch and protein present in the flour.

Viscosity and density measurements were done on the various solutions. Viscosity was measured using the size 50 Cannon-Fenske Viscometer™ (Fisher Scientific, Suwanee, GA)[7]. Density was measured using a hydrometer (Fisher Scientific, Suwanee, GA) with a range of 1.000 to 2.000.

Several tests were conducted to determine physicochemical properties of the starch. These included moisture, particle size analysis, total protein, pasting properties and elemental analysis. Moisture was measured using a programmable Ohaus™ moisture analyzer model MB45 programmed at 200 °C in 9 min; 150 °C in 3 min and 105 °C in 8 min. Particle size analysis of starch was carried out in the Coulter ™ Small Volume Module Model LS230 (Coulter® Corp., Miami, FL) particle size analyzer [1]. Based on preliminary studies, the hydrated rice starch had a maximum particle size of 10 μm. Therefore it was assumed that microfluidized rice flour slurry with particle size below 10 μm would be completely deagglomerated. Protein analysis was done on a Perkin-Elmer® Protein Analyzer (Shelton, CT) using AOCS Official Method [8]. Pasting properties of the purified starch were determined using AACC method [9]. Elemental analysis of the starch was done using Perkin Elmer 5100 Inductively-Coupled Plasma Atomic Emission Spectrometer (ICPAA) (sodium and zinc) and Graphite Furnace Atomic Absorption Spectrometer (GFAA) (cesium). The starch sample was digested by microwave heating under pressure with nitric acid in a closed Teflon® vessel. The sample was completely dissolved and subjected to ICPAA and GFAA analysis [10].

3 Results and Discussion

CeCl is one of the most expensive salts available in the market. The cheapest and least pure (>98% pure) CeCl costs more than $200/kg, but is the salt which has been routinely used for isolation of starch due to its high density (3.98, mol. Wt. 168.36) and solubility (162.22 g/

100 mL). If a laboratory was using this salt on a regular basis to screen varieties, the cost could run into thousands of dollars. NaCl is one of the cheapest salt, but its density (2.165, mol. Wt. 58.44) and solubility (35.7 g/100 mL) are relatively low as compared to CeCl. We surveyed the Handbook of Physics and Chemistry and tried to find a salt which is most dense, highly soluble, non-toxic, will not interact with starch and also is relatively cheap. $ZnSO_4 \cdot 7H_2O$ was selected because its density (1.957, mol. Wt. 287.54) was similar to that of NaCl, but it was highly soluble in water (166 g/100 mL) and the price was lower than that of CeCl (approximately $37/kg).

Complete deagglomeration of protein-starch agglomerates was the most important step in isolation of purified starch. Figs. 1 and 2 show the effect of microfluidizing 32% (db) waxy and non-waxy rice flour slurry. The control had particle size up to 400 μm. Microfluidized waxy rice flour slurry obtained using the Microfluidizer® had 90.15%±0.86 and 99.56%±0.3 of the particles less than 10 μm when passed two and four times through the Microfluidizer®, respectively. The microfluidized non-waxy rice flour slurry had 88.26%±1.24 and 99.28%±0.61 particles less than 10 μm when passed two and four times through the Microfluidizer®, respectively. Micofluidizing the slurry beyond three passes did not result in any further decrease in particle size (data not shown). But we decided to use four passes to ensure that all agglomerates below 10 μm were disintegrated.

The microfluidized slurry was centrifuged and the protein layer with the supernatant was discarded. The starch fraction was re-suspended into various density gradient system followed by rigorous washing using water. The particle size or the number of passes through the Microfluidizer® did not affect the recovery of the starch but affected the residual protein in the isolated starch (Tab. 1). Pass 2 starches had higher residual protein content than pass 4 starches and were significantly different for all different density gradient systems and type of starch (Using Fisher's LSD test at α=0.5). This was due to the presence of starch-protein agglomerates which did not

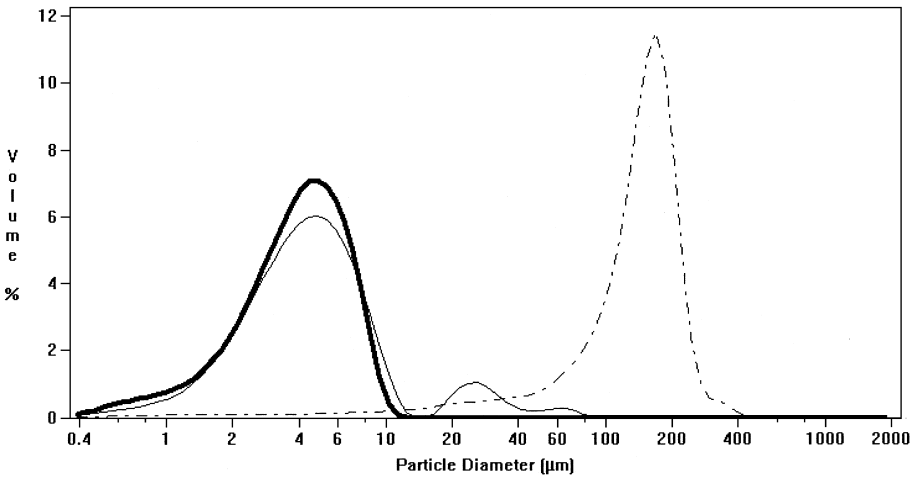

Fig. 1. Effect of microfluidizing 32% waxy rice flour slurry two and four times through the Microfluidizer®. (– · – Untreated Control; —— After 2nd pass through the Microfluidizer®; ——— After fourth pass through the Microfluidizer®).

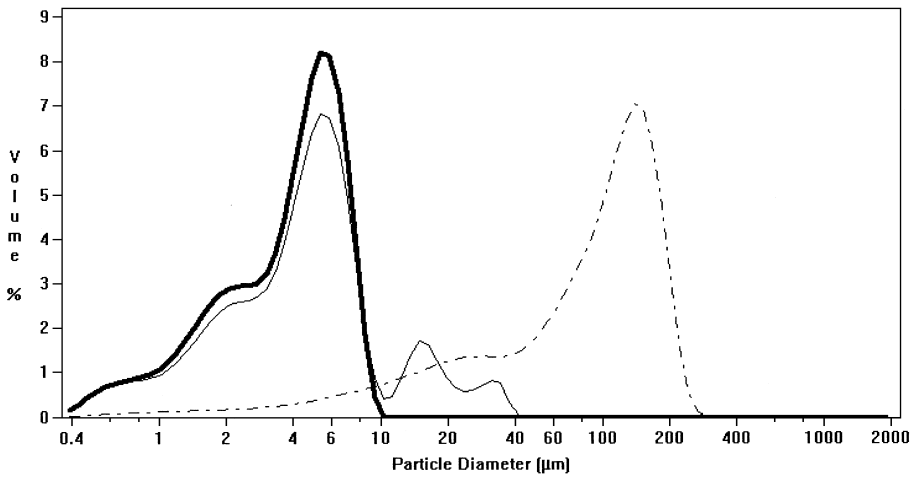

Fig. 2. Effect of microfluidizing 32% non-waxy rice flour slurry two and four times through the Microfluidizer®. (– · – Untreated Control; —— After 2nd pass through the Microfluidizer®; ——— After fourth pass through the Microfluidizer®).

Tab. 1. Effect of kinematic viscosity and density of density gradient systems on % recovery and % residual protein in starch.

Density gradient system	Kinematic viscosity [mm²/s]	Density [g/mL]	% Recovery of starch				% Protein			
			Waxy	Non-waxy	Waxy	Non-waxy	Waxy	Non-waxy	Waxy	Non-waxy
			Pass 2		Pass 4		Pass 2		Pass 4	
Water	0.97 ± 0.12	1.00	81.24	76.28	79.82	77.32	1.59 ± 0.10 (1)*	2.00 ± 0.08 (1)	1.15 ± 0.03 (1)	1.55 ± 0.03 (1)
CeCl	0.7 ± 0.00	1.60	86.51	91.20	87.96	86.32	0.48 ± 0.01 (2)	0.85 ± 0.01 (2)	0.24 ± 0.02 (2)	0.49 ± 0.01 (2)
NaCl / sucrose	14.03 ± 0.04	1.35	81.94	83.97	80.53	80.43	0.57 ± 0.10	0.88 ± 0.17 (2)	0.35 ± 0.05 (2)	0.06 ± 0.04 (3) (3)
$ZnSO_4 \cdot 7H_2O$	3.27 ± 0.06	1.40	85.08	83.91	81.21	77.53	1.10 ± 0.12 (3)	0.91 ± 0.03 (2)	0.45 ± 0.05 (4)	0.31 ± 0.11 (4)

* Similar numbers in parenthesis show that observations in various treatments for a particular group are the same (there is no significant difference between observations using the Fisher LSD Test at the 5% significance level). Different numbers within a group show that there is a significant difference between treatments.

break down after the second pass (observe particles beyond 10 μm in Figs. 1 and 2) through the Microfluidizer®. On centrifugation, larger particles settle down faster and contribute to the increased protein content of the isolated starch. After four passes (observe no particles beyond 10 μm in Figs. 1 and 2) through the Microfluidizer® all the starch-protein agglomerates disintegrated resulting in protein floating to the top and being removed during density gradient separation. This resulted in reduction of the residual protein content of the isolated starch. This indicates that the Microfluidizer® completely deagglomerates the starch in four passes through the Microfluidizer®.

Tab. 1 shows the effect of the kinematic viscosity and density of density gradient systems on the recovery of starch and residual protein in the starch. It was observed that on addition of CeCl to the water, the viscosity was reduced to 0.7 mm²/s as compared to 0.97 mm²/s for water. The system had a lower viscosity than even water. This is due to the chaobropic nature of the cesium anion [11]. Cesium chloride had the highest density of 1.60 g/mL and the lowest viscosity which resulted in highest recovery of starch with low protein content (Tab. 1). Water resulted in the lowest recovery of starch with the highest residual protein content. This is primarily due to the lower density of water as compared to other density gradient systems. Generally, all density gradient systems were satisfactory in cleaning the starch (protein <0.5%). There was a high positive correlation between % recovery of starch and density of the gradient system (Tab. 2) for both non-waxy and waxy types of starch over all passes. Therefore, higher density of the additive in the density gradient system will improve starch recovery. There was also a high negative correlation between residual protein in the purified starch and the density of the density gradient system for

Tab. 2. Correlation coefficients between % residual protein in starch and % recovery of starch against physical properties of density gradient systems and starch pasting properties.

	A	B	C	D	E	F	G	H	I	J	K
Waxy pass 2	+0.87	–0.43	–0.53	–0.41	–0.87	–0.71	–0.02	–0.81	–0.73	–0.80	–0.35
Non-waxy pass 2	+0.98	–0.01	–0.82	–0.47	–0.89	–0.70	–0.72	+0.44	–0.77	–0.88	+0.05
Waxy pass 4	+0.79	–0.40	–0.59	–0.35	–0.95	–0.52	+0.56	–0.57	+0.77	+0.54	–0.44
Non-waxy pass 4	+0.77	–0.10	–0.35	–0.64	–0.76	+0.40	–0.45	+0.43	–0.33	–0.07	+0.23

A = Recovery vs Density
B = Recovery vs Viscosity
C = Recovery vs % Protein
D = % Protein vs Viscosity
E = % Protein vs Density
F = % Protein vs Peak 1
G = % Protein vs Trough 1
H = % Protein vs Breakdown
I = % Protein vs Final Visc
J = % Protein vs Setback
K = % Protein vs Pasting Temp

all starch samples, which means that higher density of the density gradient system will result in increased purity of the starch.

This increased purity and recovery of starch was not only due to density gradient separation effects but could be also due to absorption/adsorption of these salts in the starch granule (Tab. 3). Absorption of these salts into the starch granule would make the granule heavier and result in increased settling of starch during density gradient separation. Highest recovery of starch was obtained when CeCl was used as an additive for density gradient separation (Tab.1) and CeCl also has the highest solid density (3.98) as compared to NaCl (2.165) and $ZnSO_4 \cdot 7H_2O$ (1.957). The hydrated density of rice starch and microfluidized rice protein is 1.1833 and 1.047, respectively. Since the difference between these two densities is small, separation of starch with <0.5% protein using water is not possible (Tab. 1). It seems that the salt is absorbed/adsorbed in the starch granule which increases the difference between the density of starch and protein resulting in improved separation of purified starch with high recoveries.

To determine the effect of various density gradient systems on the starch structure, pasting properties were determined using a Rapid Visco Analyser®. Generally, pasting properties of the isolated starches varied with different density gradient system (Tab. 4). No trend was observed. This could be due to a number of reasons: First the residual protein content of the isolated starch varied and therefore it could affect the pasting properties. This is especially possible for starches extracted with water which had residual protein content from 1.15 to 2.0%. Higher protein content had greater significant relationships with the pasting properties of pass 2 starches as compared to pass 4 starches (Tab. 2). Protein content had a relatively higher negative correlation to peak viscosity, final viscosity and setback viscosity for pass 2 starches as compared to pass 4 starches. Residual protein content has been shown to play a critical role in determining pasting characteristics of isolated starch, showing a negative correlation to peak viscosity of the starch paste, but a positive correlation to pasting temperature [12].

Another reason for different pasting properties of starch using different density gradient system could be the effect of % recovery of purified starch. It has been shown that starch granules isolated from the same potato but of different sizes had different physico-chemical properties including rheological properties [13, 14]. If a certain method of isolation/washing produced lower recovery of starch, smaller granules of lower density might have been lost in the supernatant which would affect pasting properties. Therefore, it is not only important to isolate purified starches but also to recover high amounts of these starches.

A third possibility is absorption/adsorption of these salts into the starch granule which could affect the pasting properties. Elemental analysis of the pass 4 purified starches using various density gradients systems showed that salts were retained in the starch granules despite rigorous washing (Tab. 3). A detailed study of salt-starch interaction and its effects on alteration of gelatinization properties is described elsewhere [15]. Cesium chloride and sodium chloride lower the breakdown viscosity of the non-waxy rice starch (Tab. 4). A similar effect of cations was found with corn starch [3]. Salts of weak bases have been known to hydrolyze starch in aqueous media [16]. The binding strengths of divalent ions with starch has been shown to be greater than those of mono-valent metal salts [17, 18]. Sodium chloride in low amounts also has been shown to inhibit the α-amylase activity [19]. This could affect the starch structural studies. Additional studies to determine the effect of the absorbed salt on debranching enzymes are being conducted. This property of absorption/adsorption of salts by the starch granule could be used to modify starches.

4 Conclusion

Density gradient systems could be used to isolate starches with low protein content from deagglomerated rice

Tab. 3. Effects of using various density gradient systems on the retention of salts in the purified starch recovered from microfluidized (4th pass) rice flour slurries.

Starch type	Element	Density gradient system			
		Water	CeCl	NaCl/sucrose	$ZnSO_4 \cdot 7H_2O$
Waxy	sodium	<10*	<10	803 ± 2.83	<10
	zinc	<10	<10	<10	937 ± 24.04
	cesium	<10	4724± 107.48	<10	<10
Non-Waxy	sodium	<10	<10	753 ± 22.63	<10
	zinc	<10	<10	<10	907.5 ± 6.36
	cesium	<10	4425 ± 7.07	<10	<10

* mg/kg starch.

Tab. 4. Effect of type (waxy and non-waxy), concentration and the number of passes of the rice flour slurry through the microfluidization process at 10.0×10^4 kPa on pasting properties of the starch fraction using the Rapid Visco Analyser.

Starch Type	Additive	Peak [RVU]*		Minimum [RVU]		Breakdown [RVU]	
		Pass 2	Pass 4	Pass 2	Pass 4	Pass 2	Pass 4
Waxy	Water	263.17 ± 0.36 (1)**	268.97 ± 1.73 (1)	161.47 ± 1.00 (1)	162.75 ± 0.60 (1)	101.69 ± 1.30 (1)	106.22 ± 2.33 (1)
	CeCl	283.31 ± 8.62 (2)	275.5 ± 10.36 (1,2)	154.78 ± 2.34 (2)	160.22 ± 2.26 (1,2)	128.53 ± 7.39 (2)	115.28 ± 12.02 (1,2)
	NaCl/sucrose	313.31 ± 2.85 (3)	280.45 ± 5.20 (2,3)	164.19 ± 3.59 (1)	158.42 ± 1.30 (1,2)	149.11 ± 5.64 (3)	122.03 ± 6.49 (2,3)
	$ZnSO_4 \cdot 7H_2O$	267.08 ± 4.48 (1)	287.53 ± 2.62 (2,3)	148.53 ± 1.13 (3)	158.36 ± 3.78 (2)	118.56 ± 4.89 (4)	129.25 ± 1.52 (3)
Non-Waxy	Water	231.81 ± 2.31 (1)	266.42 ± 4.96 (1)	134.5 ± 2.37 (1)	163.16 ± 2.79 (1)	97.30 ± 4.63 (1)	103.25 ± 2.18 (1)
	CeCl	253.91 ± 8.38 (2)	242.05 ± 0.46 (2)	200.22 ± 5.17 (2)	191.75 ± 0.72 (2)	53.69 ± 6.01 (2)	50.31 ± 0.73 (2)
	NaCl/sucrose	246.00 ± 1.25 (1,2)	243.64 ± 2.70 (2)	174.41 ± 1.91 (3)	178.58 ± 1.10 (3)	71.58 ± 1.01 (3)	65.06 ± 3.65 (3)
	$ZnSO_4 \cdot 7H_2O$	257.44 ± 13.95 (2)	275.67 ± 2.69 (3)	149.55 ± 5.96 (4)	163.86 ± 4.83 (1)	107.89 ± 10.77 (1)	111.81 ± 7.29 (4)
Starch Type	Additive	Final Visc [RVU]*		Setback [RVU]		Pasting Temp [RVU]	
		Pass 2	Pass 4	Pass 2	Pass 4	Pass 2	Pass 4
Waxy	Water	174.47 ± 2.04 (1)	181.25 ± 3.71 (1)	13.00 ± 3.04 (1)	18.50 ± 3.54 (1)	67.70 ± 0.10 (1)	67.83 ± 0.23 (1,3)
	CeCl	183.64 ± 2.38 (2)	174.11 ± 1.59 (2)	28.86 ± 0.30 (2)	13.89 ± 3.54 (1,2)	68.88 ± 0.03 (2)	69.37 ± 0.45 (2)
	NaCl/sucrose	183.47 ± 2.34 (2)	173.61 ± 1.11 (2,3)	19.28 ± 1.32 (3)	15.20 ± 1.14 (1,2)	67.25 ± 0.26 (3)	67.63 ± 0.29 (3)
	$ZnSO_4 \cdot 7H_2O$	169.00 ± 2.18 (3)	169.92 ± 0.00 (3)	20.47 ± 1.52 (3)	11.55 ± 3.78 (2)	67.88 ± 0.14 (1)	68.25 ± 0.26 (1)
Non-Waxy	Water	250.28 ± 1.84 (1)	288.22 ± 5.43 (1)	115.78 ± 1.73 (1)	125.05 ± 3.09 (1)	81.27 ± 0.19 (1,2)	81.58 ± 0.18 (1)
	CeCl	337.86 ± 6,37 (2)	319.34 ± 3.84 (2)	137.64 ± 2.87 (2)	127.58 ± 3.14 (1)	81.48 ± 0.15 (1)	81.62 ± 0.28 (1)
	NaCl/sucrose	306.08 ± 1.80 (3)	311.75 ± 2.83 (2)	131.67 ± 1.97 (3)	133.17 ± 1.97 (1)	80.77 ± 0.38 (2)	81.38 ± 0.03 (1)
	$ZnSO_4 \cdot 7H_2O$	277.33 ± 6.36 (4)	275.03 ± 3.60 (3)	127.78 ± 3.71 (3)	111.17 ± 7.81 (2)	81.62 ± 0.51 (1)	81.62 ± 0.19 (1)

* Rapid Visco Units.

** Similar numbers in parenthesis show that observations in various treatments for a particular group are the same (there is no significant difference between observations using the Fisher LSD Test at the 5% significance level). Different numbers within a group show that there is a significant difference between treatments.

flour slurry. Density gradient separation of rice starch and protein is directly dependent on the density of the gradient system/solution. A higher density of the density gradient system/solution will most likely improve starch recovery and purity. Salts are retained in the starch granules and affect the separation of protein and starch. Absorption/adsorption of heavy metal salts increases the density of the starch granule, thereby increasing the difference between density of starch and protein. This allows for efficient separation of starch with high recovery and purity. Cesium chloride resulted in highest recovery of starch. This absorption/adsorption could affect starch pasting properties and also the starch structure. Density gradient system can only be used to conduct preliminary physical studies regarding recovery and purity of starch or level of deagglomeration until more conclusive data is obtained on its effect on starch structure. Density gradient system/solution can be used to screen rice varieties coming for processing.

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